



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12P 7/62, C08G 63/06 // C12P 7/42, 7/44</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/33065</b> <b>(43) International Publication Date:</b> 7 December 1995 (07.12.95)
<b>(21) International Application Number:</b> PCT/US95/06651 <b>(22) International Filing Date:</b> 25 May 1995 (25.05.95)  <b>(30) Priority Data:</b> 08/251,828 1 June 1994 (01.06.94) US  <b>(71) Applicant:</b> THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US).  <b>(72) Inventor:</b> NODA, Isao; 1568 Hunter Road, Fairfield, OH 45014 (US).  <b>(74) Agents:</b> REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).	<b>(81) Designated States:</b> AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> PROCESS FOR RECOVERING POLYHYDROXYALKANOATES USING CENTRIFUGAL FRACTIONATION  <b>(57) Abstract</b> <p>The present invention relates to a process for recovering polyhydroxyalkanoate from a biological source material containing the polyhydroxyalkanoate, the process comprising: a) comminuting the biological source material; b) suspending the comminuted biological source material in a fluid; c) partitioning the polyhydroxyalkanoate from the other components of the biological source material by centrifugal fractionation to form a solid-solid separation; and d) recovering the polyhydroxyalkanoate.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## PROCESS FOR RECOVERING POLYHYDROXYALKANOATES USING CENTRIFUGAL FRACTIONATION

5

### TECHNICAL FIELD

The present invention relates to processes for isolating specific resin components from other biomass components. More specifically, the present invention relates to a process for the recovery of a polyhydroxyalkanoate from a biological system, such as a plant or bacteria, by using centrifugal fractionation.

### BACKGROUND

Commodity polymers are typically produced from petrochemical sources by well-known synthetic means. However, recent advances in technology have resulted in the promise of new sources of commodity polymers. Particularly promising is the production of plastic resins using living organisms ("bioplastic"), including genetically manipulated bacteria and crop plants, which are designed to produce polymers such as polyhydroxyalkanoate (PHA); a number of bacteria which naturally produce PHA are also promising sources of PHA. (see for example, NOVEL BIODEGRADABLE MICROBIAL POLYMERS, E.A. Dawes, ed., NATO ASI Series, Series E: Applied Sciences - Vol. 186, Kluwer Academic Publishers (1990); Poirier, Y., D.E. Dennis, K. Klomparens and C. Somerville, "Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants", SCIENCE, Vol. 256, pp. 520-523 (1992)). In a large scale production, for example agricultural production, the harvesting and purifying of such bioplastic from the biomass debris is a critical step for determining the practical feasibility of such technology.

The separation of polymeric lipids such as PHA from a large-scale biological source, such as an agricultural crop, is not a trivial task. The conventional separation methods used extensively in the extraction of low molecular weight lipids are not practical to employ in a resin isolation process. For example, a simple mechanical press is impractical because, unlike separating vegetable oils from oil-seeds, solid plastics cannot be squeezed out of crops by mechanical pressing.

Solvent extraction is also impractical for a number of reasons. A solution of polymer develops an extremely high viscosity, even at relatively

low concentration, thereby making the solution extremely difficult to work with. Furthermore, the stripping of solvent from polymer is a slow and difficult process. A commonly used solvent for the extraction of PHA from bacteria is chloroform. However, the use of a large amount of such a solvent, potentially harmful to health and environment if accidentally released, near the harvesting site would be undesirable.

Separation of PHA by sedimentational methods should be, in principle, possible. However, simple gravitational (1-G force) settling in a liquid suspending medium is, in fact, quite impractical. The rate of settling is extremely slow. In addition, such slow settling is easily disrupted by the Brownian motion of the fine PHA particles induced by the thermal fluctuation of the suspending fluid molecules surrounding the particles. Furthermore, the extended period of time required to settle very fine PHA particles introduces the problem of bacterial contamination and subsequent biodegradation of the particle suspension.

Based on the foregoing, there is a need for a simple and economical process for recovering bioplastics from a large-scale biological source. Such a process would preferably be easily adaptable as an integral part of the agricultural production of bioplastics.

It is therefore an object of the present invention to provide a process for recovering bioplastics from a biological source material.

#### SUMMARY

The present invention relates to a process for recovering polyhydroxyalkanoate from a biological source material containing the polyhydroxyalkanoate, the process comprising: a) comminuting the biological source material; b) suspending the comminuted biological source material in a fluid; c) partitioning the polyhydroxyalkanoate from the other components of the biological source material by centrifugal fractionation to form a solid-solid separation; and d) recovering the polyhydroxyalkanoate.

#### DETAILED DESCRIPTION

The present invention answers the need for a process for recovering bioplastics from a biological source material.

The following is a list of definitions for terms used herein.

"g/sec" means grams per second.

"g/min" means grams per minute.

"μ" means micron(s).

"psi" means pounds per square inch.

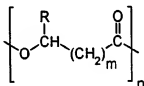
"MPa" means mega pascal, which is equivalent to about 145 psi.

"Fractionation" means the separation and/or isolation of components of a mixture. The invention described herein preferably achieves such fractionation due to differences in the density and/or particle size of the various components.

- 5 "Solid-solid separation" and "solid-solid fractionation" mean the separation or partitioning of components in a sample wherein each fraction comprises a component in a solid state. For example, a separation resulting in a fraction comprising PHA granules suspended in a fluid medium and a fraction comprising other insoluble biomass is considered a
- 10 solid-solid separation.

- "Liquid-solid separation" and "liquid-solid fractionation" mean the separation or partitioning of components in a sample wherein at least one fraction contains an otherwise solid component in liquid state, and at least one fraction comprises a component in a solid state. For example, a
- 15 separation resulting in a fraction comprising PHA dissolved in a solvent and a fraction comprising other insoluble biomass is considered a liquid-solid separation.

"Polyhydroxyalkanoate" and "PHA" mean a polymer having the following general structure:



- 20 wherein R is preferably an alkyl or alkenyl, m is 1 or 2, and n is an integer. The structure enclosed in brackets is commonly referred to as a repeating unit. The terms polyhydroxyalkanoate and PHA include polymers containing one or more different repeating units. Examples of preferred
- 25 PHAs recoverable by the present process included those disclosed in U.S. Patent Application Ser. No. 08/187,969, Noda, filed January 28, 1994; U.S. Patent Application Ser. No. 08/188,271, Noda, filed January 28, 1994; U.S. Patent Application Ser. No. 08/189,029, Noda, filed January 28, 1994; and European Patent Application Ser. No. 533 144, Shiotani and Kobayashi,
- 30 published March 24, 1993.

- "Recovering polyhydroxyalkanoate from a biological source material", in addition to referring to the recovery of the particular PHA produced by a biological source material which produces a single PHA, also refers to the recovery of one or more types of PHA when the biological source material
- 35 produces more than one type of PHA.

"Alkyl" means a carbon-containing chain which may be straight, branched or cyclic, preferably straight; substituted (mono- or poly-) or unsubstituted; and saturated.

- 5 "Alkenyl" means a carbon-containing chain which may be straight, branched or cyclic, preferably straight; substituted (mono- or poly-) or unsubstituted; and monounsaturated (i.e., one double or triple bond in the chain), or polyunsaturated (i.e., two or more double bonds in the chain, two or more triple bonds in the chain, or one or more double and one or more triple bonds in the chain).

- 10 "Comprising" means that other steps and other ingredients which do not affect the end result can be added. This term encompasses the terms "consisting of" and "consisting essentially of".

All percentages are by weight of total composition unless specifically stated otherwise.

- 15 The present invention relates to a process for recovering (i.e., isolating) polyhydroxyalkanoate from a biological source material containing the polyhydroxyalkanoate, the process comprising centrifugal fractionation of the biological source material such that the polyhydroxyalkanoate is partitioned from the other components of the biological source material by  
20 solid-solid separation.

- PHA components found in biological systems (e.g., conventional, or genetically engineered bacteria or genetically engineered plants) tend to settle at rates different from other cellular components such as proteins and carbohydrates when they are suspended in a common fluid medium. The  
25 difference in the sedimentation rate results in the spatial segregation of mixed particulates into multiple layers (fractionation) each containing predominantly a single component.

- According to the Stokes' law of sedimentation, there are two major factors affecting the sedimentation rates of particles: differences in the  
30 density and size of suspended particles. These factors independently control the separation efficiency of the fractionation process.

- Density is an intrinsic property of the material to be separated. Generally, there is no easy way of manipulating the density of an individual component to be fractionated. The density difference between particles in a  
35 composition is not always large enough to achieve substantial fractionation of one component from the rest. When the densities of the components are sufficiently different, a high degree of separation can be achieved, particularly when a suspending medium having an intermediate density is

used. The density of PHA is sufficiently different from other biomass components, such as proteins and carbohydrates, to achieve some degree of fractionation.

- Differences in particle size also contribute greatly to the effectiveness of the fractionation process. PHA is generally stored in biological systems in the form of very fine granules having a diameter of about or below 1  $\mu$ . The particle size of PHA granules are therefore much smaller as compared to other cellular components from the disrupted cell. In addition, particle size can be further manipulated by a post-harvest processing which includes grinding or colloid-milling. Specific types of biological source material and the process are discussed in more detail below.

#### Biological Source Material

- Sources from which PHA is recovered via the process of the present invention include single-cell organisms such as bacteria or fungi and higher organisms such as plants (herein collectively referred to as "biological source material" or "BSM"). While such BSM could be wild-type organisms, they are preferably genetically manipulated species specifically designed for the production of a specific PHA of interest to the grower. Such genetically manipulated organisms are produced by incorporating the genetic information necessary to produce PHA. Typically, such genetic information is derived from bacteria which naturally produce PHA.

- Plants useful in the present invention include any genetically engineered plant designed to produce PHA. Preferred plants include agricultural crops such cereal grains, oil seeds and tuber plants; more preferably, avocado, barley, beets, broad bean, buckwheat, carrot, coconut, copra, corn (maize), cottonseed, gourd, lentils, lima bean, millet, mung bean, oat, oilpalm, peas, peanut, potato, pumpkin, rapeseed (e.g., canola), rice, sorghum, soybean, sugarbeet, sugar cane, sunflower, sweetpotato, tobacco, wheat, and yam. Such genetically altered fruit-bearing plants useful in the process of the present invention include, but are not limited to apple, apricot, banana, cantaloupe, cherries, grapes, kumquat, lemon, lime, orange, papaya, peaches, pear, pineapple, tangerines, tomato, and watermelon. Preferably the plants are genetically engineered to produced PHA pursuant to the methods disclosed in Poirier, Y., D.E. Dennis, K. Klomprens and C. Somerville, "Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants", SCIENCE, Vol. 256, pp. 520-523 (1992); U.S. Patent Application Ser. No. 08/108,193, Somerville, Nawrath and Poirier, filed August 17, 1993; and U.S. Patent Application Ser.

No. 07/732,243, Somerville, Poirier and Dennis, filed July 19, 1991. Particularly preferred plants are soybean, potato, corn and coconut plants genetically engineered to produce PHA.

- Bacteria useful in the present invention include any genetically engineered bacteria designed to produce PHA, as well as bacteria which naturally produce PHA. Examples of such bacteria include those disclosed in NOVEL BIODEGRADABLE MICROBIAL POLYMERS, E.A. Dawes, ed., NATO ASI Series, Series E: Applied Sciences - Vol. 186, Kluwer Academic Publishers (1990); US Patent 378,155, Peoples and Sinskey, issued December 27, 1989; US Patent 5,250,430, Peoples and Sinskey, issued October 5, 1993; US Patent 5,245,023, Peoples and Sinskey, issued September 14, 1993; US Patent 5,229,279, Peoples and Sinskey, issued July 20, 1993; and US Patent 5,149,644, Lubitz, issued September 22, 1992.

- It is preferable that the BSM contain a sufficient quantity of PHA to make the process economically desirable. Preferably, the initial content of PHA in the source material should be at least about 5% of the total dry weight; more preferably at least about 25%; more preferably at least about 50%; more preferably still, at least about 75%.

#### Isolation Process

- The process of the present invention preferably involves the following unit-operation steps: pretreatment, size reduction, suspension, and centrifugal separation. The optimal range of unit operation conditions or individual devices will vary considerably according to the type of raw BSMs used.

- The pretreatment of source materials comprising PHA is preferred in order to remove low molecular weight contaminants readily soluble in appropriate solvents, such as sugars, oils and sometimes moisture. A variety of standard pretreatment methods used for the processing of food crops are known to those skilled in the art and may be readily employed for the pretreatment step of the present invention. Examples of such pretreatment steps include oil extraction (for example, see BAILEY'S INDUSTRIAL OIL AND FAT PRODUCTS, THIRD ED., John Wiley and Sons: New York (1964), pp. 663-713), water washing (for example, see US Patent 2,881,076, Sair, issued April 7, 1959), and alcohol washing (for example, see Eldridge, A.C., W.J. Wolf, A.M. Nash and A.K. Smith, "Alcohol Washing of Soybean Protein", AGRICULTURAL AND FOOD CHEMISTRY, July-August, 1963, pp. 323-328).



The pretreated material comprising PHA granules is then pulverized (e.g., dry milling) to small fragments, by first using a common vibratory or hammer mill. The pulverization of source material by dry milling, particularly for agricultural crop plants comprising PHA granules, preferably produces grain flour of a particle size finer than about 500  $\mu$  in diameter, more preferably finer than about 300  $\mu$ , more preferably still, finer than about 100  $\mu$ .

The comminuted material is then dispersed in a suspending fluid such as water, chlorinated carbon solvents (e.g., chloroform, carbon tetrachloride, or dichloroethane), various organic solvents (e.g., ethanol, methanol, acetone, methyl ethyl ketone, ethyl acetate, hexane, heptane, pentane, or mixtures thereof), or supercritical fluids (e.g., carbon dioxide or nitrous oxide); preferably water. If the suspending fluid is water, the water is preferably heated to promote hydration of certain non-PHA components prior to the wet milling to a temperature preferably not exceeding 90°C. If a suspending medium having an intermediate density between the PHA and other biomass is desired, then the suspending medium is preferably an aqueous solution of an organic or inorganic salt, or an aqueous solution of a water-soluble sugar. Useful organic salts include, but are not limited to, potassium glycolate, potassium citrate, potassium lactate, potassium malate, and dipotassium tartrate. Useful inorganic salts include, but are not limited to, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, and calcium sulfate. Useful water-soluble sugars include, but are not limited to, sucrose, glucose, and raffinose.

The material is further treated by wet milling using a device such as a colloid mill, sonicator, or homogenizer, to obtain the desired particle size distribution for the dispersed solids. For wet milling, the comminuted material is suspended in a fluid. Preferred fluids include, but are not limited to water; ethanol; and aqueous solutions of organic salts, inorganic salts or sugars. The wet milling should preferably produce suspensions having an average particle size of smaller than about 50  $\mu$ .

The suspension mixture of PHA and other biomass components is then processed with a centrifugal device to fractionate PHA-rich particles from the other biomass. Preferred centrifugal devices include, but are not limited to, centrifuge, ultracentrifuge, or hydrocyclone separator. An industrial scale centrifugal separation device may be used in the process of the present invention for fractionation of the PHA, so long as the device provides sufficient "G" force (i.e., sedimentational force field created, for

example, by centrifugal effect expressed in terms of the equivalent to gravitational force) to achieve the sedimentation of solid particles above the rate necessary to overcome the Brownian motion of particles to be settled. The centrifugal force field employed in the process of the present invention is preferably at least about 10 G (i.e., ten times faster than simple gravimetric settling) to achieve rapid and efficient fractionation of PHA from other biomass components. More preferably, the centrifugal force field is at least about 100 G, more preferably at least about 1,000 G, more preferably at least about 10,000 G, more preferably still at least about 100,000 G.

In one embodiment of the present invention, a hydrocyclone is employed as the centrifugal device. A hydrocyclone consists of a conical cavity with an eccentric inlet port and two exit ports above and below the conical cavity. The vortex flow created by the off-centered high-speed injection of fluid creates a centrifugal force resulting in the sedimentation of heavy particles toward the inner wall of the conical cavity. The heavier portion will exit predominantly from the smaller tip of the cone, while the lighter portion will exit from the wider part of the cone. The centrifugal force field increases in a hydrocyclone with the inlet feed rate and decreases with the diameter of the conical cavity. For example, in a typical 1 cm diameter hydrocyclone, it is possible to achieve well above 100 G of centrifugal force by maintaining the feed rate of suspension above several tens of gallons per minute. (See, for example, Day, R.W., "Hydrocyclones in Process and Pollution Control", CHEMICAL ENGINEERING PROGRESS, Vol. 69, pp. 67-72 (1973); and US Patent 2,754,968, Vegter and Hage, issued July 17, 1956; and KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY, THIRD ED., Vol. 12, pp. 1-29, John Wiley and Sons, (1990)). Hydrocyclones useful in the present invention are available from a variety of manufacturers including Dorr-Oliver, Inc. (Milford, CT), Yardney Water Management Systems, Inc. (Riverside, CA), and Quality Solids Separation Co. (Houston, TX).

In another embodiment of the present invention, a continuous centrifuge may be employed as the centrifugal device. A continuous centrifuge comprises a rapidly rotating cylinder having a feed suspension flowing inside the cylinder. As a result of the rotation, centrifugal force is created thereby promoting sedimentation of heavier components toward the inner wall of the rotating cylinder. The sedimentate is continuously collected by a scraping mechanism while the supernatant is removed as effluent. A typical industrial scale centrifuge operating at several thousand rpm can easily produce a centrifugal force well above 100 G. (See, for

example, Ambler, C.M., "The Evaluation of Centrifuge Performance", CHEMICAL ENGINEERING PROGRESS, Vol. 48, pp. 150-158, (1952); and KIRK-OTHEMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY, THIRD ED., Vol. 12, pp. 1-29, John Wiley and Sons, (1990)). Centrifuges useful in the present  
5 invention are available from a variety of manufacturers, including Humboldt Decanter, Inc. (Atlanta, GA), Western States Machine Co. (Hamilton, OH), and Bird Machine Co. (South Walpole, MA).

Preferably, the process of the present invention yields at least about 70% of the PHA in the source material, more preferably at least about 80%,  
10 more preferably still at least about 90%.

Preferably, at least about 85% of the dry mass of the PHA-rich fraction isolated by the process of the present invention is PHA, more preferably at least about 95%, more preferably still at least about 99%.

The PHAs recovered by the process of this invention are useful for forming a variety of plastic articles, including those disclosed in US Patent Application Ser. No. 08/187,969, Noda, filed January 28, 1994; US Patent Application Ser. No. 08/188,271, Noda, filed January 28, 1994; and US Patent Application Ser. No. 08/189,029, Noda, filed January 28, 1994. Such plastic articles include, but are not limited to, films, sheets, foams,  
20 fibers, nonwovens, elastomers, adhesive and molded articles. Such plastic articles can be further incorporated into a variety of useful products including, but not limited to, personal cleansing wipes; disposable health care products such as bandages, wound dressings, wound cleansing pads, surgical gowns, surgical covers, surgical pads; other institutional and health  
25 care disposables such as gowns, wipes, pads, bedding items such as sheets and pillowcases, foam mattress pads.

The following non-limiting examples illustrate the methods of the present invention.

#### EXAMPLE 1

##### 30 Isolation of Poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) from Soybeans

Soybeans, from a genetically altered soybean plant, comprising poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) are roll milled to form thin flakes. The low molecular weight lipids and oils contained in the flakes are initially removed by pressing the flakes. The remaining low molecular lipids  
35 and oils are subsequently extracted from the flakes by using hexane as a solvent. The resulting defatted soybean flakes are dried and pulverized using a vibratory energy mill (Sweco, Florence, KY) to produce a flour having an average particle size of less than 80  $\mu$ . The flour is then hydrated

- in water at 65°C for 30 minutes to produce a suspension containing 7% solids by weight. The suspension is then passed through a colloid mill (Littleford Day, Florence, KY) once to assure complete mixing. The suspension is then passed through a homogenizer (Model 3M, APV Gaulin, Willimington, MA) operated at 8,000 psi, two times, to produce a suspension mixture consisting of fine granules of polymer having an average particle size of less than 1  $\mu$  and other soybean biomass debris comprising proteins and carbohydrates. The homogenized suspension of soybeans is fed to a hydrocyclone (DOXIE TYPE-A, Dorr-Oliver, Milford, CT) with a heavy duty pump (Model 4678-10S, Northern Pump, Minneapolis, MN) at a feed rate of 150 g/sec under a nominal pressure of 3 MPa. The effluent stream coming out the top section of the hydrocyclone contains most of the polymer granules. This portion of the suspension is spray dried and washed with 40% water / 60% ethanol mixture to remove soluble residual components such as sugars, to produce a cake of poly(3-hydroxybutyrate-co-hydroxyoctanoate) granules with a purity of greater than 95%, and a yield of about 85% with respect to the starting material.

#### EXAMPLE 2

##### Isolation of Poly(3-hydroxybutyrate-co-hydroxyhexanoate) from Maize

- Grains of maize (corn), from a genetically altered maize plant, comprising poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) are hammer milled to form meals. The low molecular weight lipids and oils contained in the meals are removed first by pressing the flakes and are then further extracted by using hexane as the solvent and washed with 40% water / 60% ethanol mixture to remove other soluble components such as sugars. The resulting defatted and desugared maize meals are dried and comminuted using a vibratory energy mill (Sweco, Florence, KY) to produce a flour having an average particle size of less than 80  $\mu$ . The flour is then hydrated in water at 65°C for 30 min to produce a suspension containing 7% solids by weight. The suspension is then passed through a colloid mill (Littleford Day, Florence, KY) once to assure complete mixing. The suspension is then passed twice through a homogenizer (Model 3M, Gaulin, Willmington, MA) operated at 8,000 psi to produce a suspension mixture consisting of fine granules of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) having an average particle size of less than 1  $\mu$  and other maize biomass debris comprising proteins and carbohydrates. The homogenized suspension of soybeans is fed to a continuous centrifuge (6" Solid Bowl Centrifuge, Bird Machine Co., South Walpole, MA) at a feed rate of 1,500 g/min. The



- through a homogenizer (Model 3M, Gaulin, Wilmington, MA) operated at 8,000 psi to produce a suspension mixture consisting of fine granules of poly(3-hydroxybutyrate-co-3-hydroxydecanoate) having an average particle size of less than 1  $\mu$  and other coconut biomass debris. The homogenized
- 5 suspension of coconuts is fed to a hydrocyclone (Doxie Type-A, Dorr-Oliver, Milford, CT) with a heavy duty pump (Model 4678-10S, Northern Pump, Minneapolis, MN) at a feed rate of 200 g/sec under a nominal pressure of 4 MPa. The effluent stream coming out the top section of the hydrocyclone will contain most of the poly(3-hydroxybutyrate-co-3-hydroxydecanoate)
- 10 granules. This portion of the suspension is spray dried to produce a cake of poly(3-hydroxybutyrate-co-3-hydroxydecanoate) granules with a purity higher than 95%, and a yield of about 85% with respect to the starting material.

#### EXAMPLE 5

- 15 Isolation of Poly(3-hydroxybutyrate-co-3-hydroxyheptanoate) from Potatoes

- Potato flakes, from potatoes obtained from a genetically altered potato plant, comprising poly(3-hydroxybutyrate-co-3-hydroxyheptanoate) are washed with water and then hydrated at 65°C for 30 min to produce a suspension containing 7% solids by weight. The suspension is then passed
- 20 through a colloid mill (Littleford, Day, Florence, KY) once to assure complete mixing. The suspension is then passed twice through a homogenizer (Model 3M, Gaulin, Wilmington, MA) operated at 8,000 psi to produce a suspension mixture consisting of fine granules of poly(3-hydroxybutyrate-co-3-hydroxyheptanoate) having an average particle size
- 25 of less than 1  $\mu$  and other potato biomass debris. The homogenized suspension of potato is fed to a continuous centrifuge (6" Solid Bowl Centrifuge, Bird Machine Co., South Walpole, MA) at a feed rate of 1,500 g/min. The supernatant effluent stream coming out of the continuous centrifuge is spray dried to produce a cake of poly(3-hydroxybutyrate-co-3-
- 30 hydroxyheptanoate) granules with a purity higher than 95%, and a yield of about 85% with respect to the starting material.

#### EXAMPLE 6

##### Isolation of Poly(3-hydroxybutyrate) from *A. eutrophus*

- A culture of *A. eutrophus* which naturally produces poly(3-
- 35 hydroxybutyrate) is treated with an ultrasonic sonicator (Branson Ultrasonics Corp., Danbury, CT) to produce a suspension mixture consisting of fine granules of poly(3-hydroxybutyrate) having an average particle size of less than 1  $\mu$  and other bacterial biomass debris containing about 20%

solids by weight. The homogenized suspension of bacterial components is fed to a hydrocyclone (Doxie type-A, Dorr-Oliver, Milford, CT) with a heavy duty pump pressure of 4 MPa. The effluent stream coming out the top section of the hydrocyclone contains most of the poly(3-hydroxybutyrate) granules. This portion of the suspension is spray dried to produce a cake of poly(3-hydroxybutyrate) granules with a purity higher than 95%, and a yield of about 90% with respect to the starting material.

#### EXAMPLE 7

##### Isolation of Poly(3-hydroxybutyrate) from *E. coli*

- 10 A culture of *E. coli* which has been genetically manipulated to produce poly(3-hydroxybutyrate) is treated with an ultrasonic sonicator (Branson Ultrasonics Corp., Danbury, CT) to produce a suspension mixture consisting of fine granules of poly(3-hydroxybutyrate) having an average particle size of less than 1  $\mu$  and other bacterial biomass debris containing about 5% solids by weight. The homogenized suspension of bacterial components is fed to a hydrocyclone (Doxie type-A, Dorr-Oliver, Milford, CT) with a heavy duty pump pressure of 4 MPa. The effluent stream coming out the top section of the hydrocyclone contains most of the poly(3-hydroxybutyrate) granules. This portion of the suspension is spray dried to produce a cake of poly(3-hydroxybutyrate) granules with a purity higher than 95%, and a yield of about 90% with respect to the starting material.

All publications and patent applications mentioned hereinabove are hereby incorporated in their entirety by reference.

- 25 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to one skilled in the art and are to be included in the spirit and purview of this application and scope of the appended claims.

What is claimed is:

1. A process for recovering polyhydroxyalkanoate from a biological source material containing the polyhydroxyalkanoate, the process characterized in that it comprises:
  - a. comminuting the biological source material;
  - b. suspending the comminuted biological source material in a fluid; and
  - c. partitioning the polyhydroxyalkanoate from the other components of the biological source material by centrifugal fractionation to form a solid-solid separation; and
  - d. recovering the polyhydroxyalkanoate.
2. The process of Claim 1, wherein the biological source material is plant material.
3. The process of Claim 2, wherein the biological source material is avocado, barley, beets, broad bean, buckwheat, carrot, coconut, copra, corn, cottonseed, gourd, lentils, lima bean, millet, mung bean, oat, oilpalm, peas, peanut, potato, pumpkin, rapeseed, rice, sorghum, soybean, sugarbeet, sugar cane, sunflower, sweetpotato, tobacco, wheat, yam, apple, apricot, banana, cantaloupe, cherries, grapes, kumquat, lemon, lime, orange, papaya, peaches, pear, pineapple, tangerines, tomato, or watermelon.
4. The process of Claim 3, wherein the biological source material is soybean.
5. The process of Claim 1, wherein the biological source material is bacteria.
6. The process of any of Claims 1-5, wherein the centrifugal fractionation is carried out by a hydrocyclone.
7. The process of any of Claims 1-5, wherein the centrifugal fractionation is carried out by centrifugation.
8. The polyhydroxyalkanoate recovered by the process of any of Claims 1-5.
9. The polyhydroxyalkanoate recovered by the process of Claim 1, wherein the centrifugal fractionation is carried out by a hydrocyclone.



10. The polyhydroxyalkanoate recovered by the process of Claim 1, wherein the centrifugal fractionation is carried out by centrifugation.

## INTERNATIONAL SEARCH REPORT

Internat. J. Application No  
PCT/US 95/06651

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12P/62 C08G63/06 //C12P7/42, C12P7/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 3 275 610 (MOBIL OIL CORP.) 27 September 1966 see the whole document ---	1-10
P, X	EP, A, 0 622 462 (REPSOL QUIMICA S.A.) 2 November 1994	1, 5-10
A	see the whole document ---	2-4
X	EP, A, 0 533 144 (KANEGAFUCHI KAGAKU KOGYO) 24 March 1993 cited in the application	8-10
A	see page 8 - page 9; claims 1-13 ---	1-7
	--- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "A" document member of the same patent family

Date of the actual completion of the international search

29 August 1995

Date of mailing of the international search report

03.10.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040; Tlx. 31 651 epo nl,  
Fax: (+ 31-70) 340-3016

Authorized officer

Douschan, K

## INTERNATIONAL SEARCH REPORT

Internat. Application No.

PCT/US 95/06651

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCIENCE, vol. 256, 24 December 1992 pages 520-523, POIRIER, Y. ET AL. 'Polyhydroxybutyrate, a Biodegradable Thermoplastic, Produced in Transgenic Plants'	8-10
A	cited in the application see the whole document  -----	1-7

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 95/06651

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-3275610	27-09-66	NONE	
EP-A-622462	02-11-94	ES-A- 2062955	16-12-94
EP-A-533144	24-03-93	JP-A- 5093049	16-04-93
		US-A- 5292860	08-03-94